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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of Kathleen C.M. Campbell
Art Unit 1614
Serial No. 09/911,195
Filed July 23, 2001
Confirmation No. 2942
For THERAPEUTIC USE OF D-METHIONINE TO REDUCE THE TOXICITY OF NOISE
Examiner Rebecca Cook

DECLARATION OF KATHLEEN C. M. CAMPBELL UNDER 37 CFR 1.132

TO THE COMMISSIONER OF PATENTS AND TRADEMARKS,

SIR:

I, Kathleen C.M. Campbell, hereby declare and state as follows:

1. I reside at 11941 Clearspring Drive, Glenarm, Illinois 62536.

2. I received a Doctor of Philosophy in Audiology/Hearing Science from the University of Iowa in 1989.

3. I am currently a Professor and the Director of Audiology Research in the Division of Otolaryngology, Department of Surgery at the Southern Illinois University School of Medicine in Springfield, Illinois.

4. I am the named inventor of the subject application, which claims methods for treating or preventing ototoxicity in a patient exposed to noise for a time and at an intensity sufficient to result in ototoxicity.

5. An experimental study was conducted under my supervision to evaluate the effect of administering D-methionine to experimental animals that were exposed

to noise sufficient to induce ototoxicity. The design of this study comprised two independent groups of subjects, where one group was used as the control and the other group received the treatment. The subjects were male adult *chinchilla laniger* divided equally into two experimental groups. The two groups consisted of a saline control group and a post-noise D-Met treatment group. Baseline hearing thresholds obtained through auditory brainstem response (ABR) were taken before the initial noise exposure and at 21 days after noise exposure for each subject.

6. ABR testing was performed using an Intelligent Hearing Systems evoked potential unit. Subcutaneous electrodes were placed at the vertex (non-inverting), to a point directly below the ipsilateral pinna (inverting) with a ground electrode in the hind leg. For measurement of the ABR threshold, animals were fully anesthetized throughout all ABR procedures with 1ml/mg intramuscular injection of Rompun cocktail, which is a solution containing 86.21 mg/kg ketamine and 2.76 mg/ml Xylazine. This initial injection was supplemented as needed with half doses. Stimuli consisted of tone bursts (1 ms Blackman rise/fall ramp, 0 ms plateau) at octave intervals 2, 4, 6, and 8 kHz. All acoustic stimuli were routed through a computer-controlled attenuator to an insert earphone (Etymotic Research). The sound delivery tube of the insert earphone was positioned approximately 5 mm from the tympanic membrane. Earphone sound delivery was calibrated using a coupler attached to a sound level meter approximating the distance from the earphone to the tympanic membrane. Five hundred samples were collected from the recording electrode, at a rate of 10/s. An intensity series was obtained for each animal starting at 100 dB SPL and then in 10-dB descending steps. Threshold was defined as the lowest level eliciting a replicable, visually detectable response. All ABR testing was conducted in a double walled sound booth.

7. Prior to being exposed to noise, the animals were housed in the Southern Illinois University School of Medicine Laboratory Animal Care Facility (LAM) for a minimum of 5 days. The animals were acclimated to the wire cages and sound exposure booth prior to the noise exposure. All noise exposure was administered inside a sound booth housed in LAM. The sound booth isolated the noise exposures from the outside environment. Our noise exposure protocol was developed from the procedure of Hu et al., Hear. Res. 113:198-206 (1997). Specifically, an octave band noise centered at 4 kHz was generated by a TDT GNS 40X white noise generator routed through an attenuator (TDT PA3), a filter (Krohn-Hite 3384) and a power amplifier (Sony 55ES) to a custom built acoustic exponential horn with a maximum output at 4kHz using an Altec 290E driver. The loudspeaker was suspended directly above the cage with the nozzle feeding into the cage, giving the animals access to water during the noise exposure period. Each animal was exposed to noise at a level of 105 dB SPL for 6 hours. During the noise exposure, the animal was unrestrained in a small wire cage with ad-lib water access. When the animals were not being exposed to noise, they were housed in a quiet animal colony.

8. Animals received one dose of D-Met (Acros; 200mg/kg) or saline by intraperitoneal injection starting 1 hour post noise exposure for one dose plus 4 additional doses BID (5 doses). Auditory threshold shifts were calculated (see data) as post-noise thresholds (dB SPL) ***minus*** baseline threshold (dB SPL). Means were plotted as a function of treatment group (saline-noise and D-Met antioxidant post-treatment), over time (zero, 21 days) and by threshold test frequency. The statistical analysis consisted of Wilcoxon's rank sum test on the difference scores between the two time points, for each of the four frequencies separately. The analysis was performed twice to examine the effect, if any, of the use of both ears from the same subject in a subset of the data. Thus, the sample size was 10 subjects per group in each analysis. The results of the two

separate analyzes were virtually identical. Statistical significance was set at the 5 percent level. The raw data and a graph summarizing these results are in attachment A.

9. From the experimental results described in this declaration, I have shown that the treatment regimen used in the experiments was effective to significantly reduce the threshold shift at each frequency tested (e.g., 2 kHz, 4, kHz, 6 kHz and 8 kHz) in the subjects at 21 days after noise exposure. The experimental group had significantly smaller ABR threshold shifts than the control group at each frequency tested including 2, 4, 6, and 8 kHz. The summary graph shows means and standard error bars for the ABR threshold shifts for each frequency tested for the control and experimental groups. The stars on the graph indicate that the change was significant at the .05 level or better for that particular measure.

10. I hereby declare and state that all statements made herein are to my own knowledge true; and that all statements made on information and beliefs are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements will jeopardize the validity of the above-identified application or any patent issued thereon.

Kathleen C.M. Campbell

Kathleen C.M. Campbell

July 1, 2005

Date

SIU 7396
PATENT

Attachment A

Data for control group:

Group	Animal		2K	4K	6K	8K
Saline 0 Hrs	E1[left ear]	Baseline dB	20	30	30	30
		21 day dB	60	80	60	80
		Threshold shift	40	50	30	50
	E1[right ear]	Baseline dB	20	30	20	30
		21 day dB	80	80	60	90
		Threshold shift	60	50	40	60
	E2[left ear]	Baseline dB	10	20	10	20
		21 day dB	20	20	10	20
		Threshold shift	10	0	0	0
	E2[right ear]	Baseline dB	20	10	10	0
		21 day dB	20	10	10	20
		Threshold shift	0	0	0	20
	E3[left ear]	Baseline dB	10	20	30	10
		21 day dB	40	50	30	20
		Threshold shift	20	30	0	10
	E3[right ear]	Baseline dB	20	10	20	20
		21 day dB	40	40	20	20
		Threshold shift	20	30	0	0
	E4[left ear]	Baseline dB	20	10	0	10
		21 day dB	20	20	10	10
		Threshold shift	0	10	10	0
	E4[right ear]	Baseline dB	20	20	0	10
		21 day dB	20	70	10	20
		Threshold shift	0	50	10	10
	E5[left ear]	Baseline dB	20	20	10	20

SIU 7396
PATENT

		21 day dB	40	30	20	20
		Threshold shift	20	10	10	0
	E5[right ear]	Baseline dB	30	20	10	30
		21 day dB	50	40	20	30
		Threshold shift	20	20	10	0

Data for D-met treatment group:

Group	Animal		2K	4K	6K	8K
D-Met 1 Hr	E21[left ear]	Baseline dB	20	30	20	30
		21 day dB	30	40	20	30
		Threshold shift	10	10	0	0
	E21[right ear]	Baseline dB	30	30	30	30
		21 day dB	40	30	20	30
		Threshold shift	10	0	-10	0
	E22[left ear]	Baseline dB	20	10	20	30
		21 day dB	20	10	20	30
		Threshold shift	0	0	0	0
	E22[right ear]	Baseline dB	20	10	20	30
		21 day dB	20	10	20	30
		Threshold shift	0	0	0	0
	E23[left ear]	Baseline dB	20	20	20	30
		21 day dB	20	20	20	30
		Threshold shift	0	0	0	0
	E23[right ear]	Baseline dB	20	20	20	30
		21 day dB	20	30	20	20
		Threshold shift	0	10	0	-10
	E25[left ear]	Baseline dB	30	20	20	30
		21 day dB	30	20	20	30
		Threshold shift	0	0	0	0
	E25[right ear]	Baseline dB	30	20	10	20
		21 day dB	30	20	10	20
		Threshold shift	0	0	0	0
	E29[left ear]	Baseline dB	30	30	20	20

		21 day dB	30	30	20	20
		Threshold shift	0	0	0	0
	E29[right ear]	Baseline dB	30	20	10	20
		21 day dB	30	20	10	20
		Threshold shift	0	0	0	0

Data for control group:

Group	Animal		2K	4K	6K	8K
Saline 0 Hrs	CON 1	Baseline dB	30	20	10	10
		21 day dB	40	30	0	10
		Threshold shift	10	10	-10	0
	CON 2	Baseline dB	30	30	0	0
		21 day dB	40	50	10	0
		Threshold shift	10	20	10	0
	CON 3	Baseline dB	30	30	0	10
		21 day dB	40	50	20	20
		Threshold shift	10	20	20	10
	CON 4	Baseline dB	20	20	0	10
		21 day dB	40	50	0	20
		Threshold shift	20	30	0	10
	CON 5	Baseline dB	30	30	20	20
		21 day dB	70	60	30	50
		Threshold shift	40	30	10	30

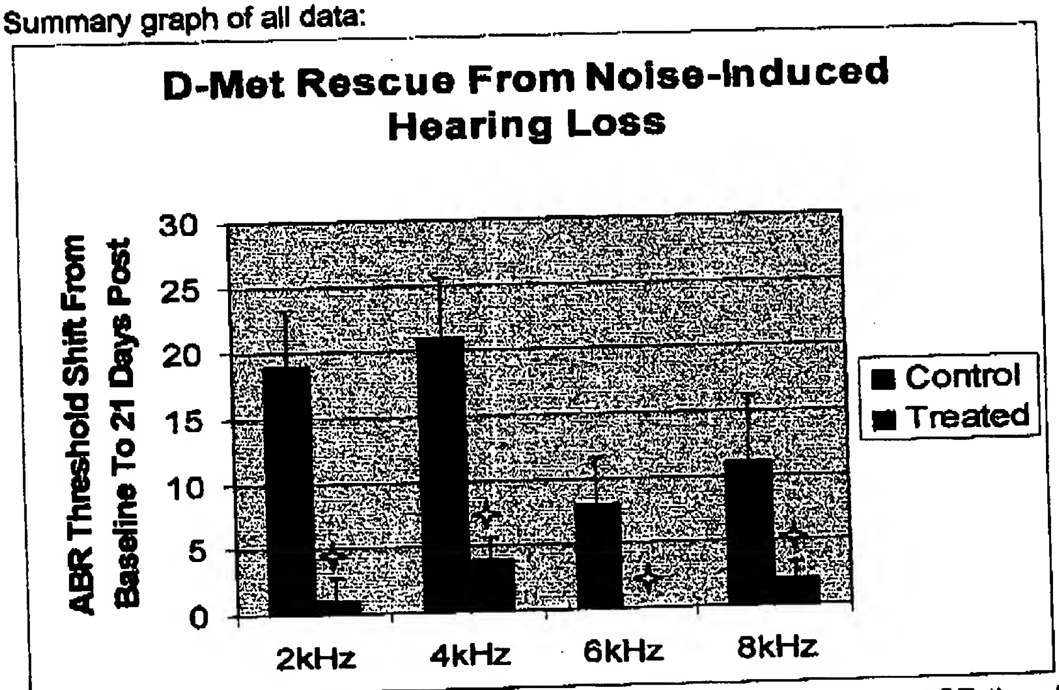
Data for D-met treatment group:

Group	Animal		2K	4K	6K	8K
D-Met 1 Hr	5 MET 1	Baseline dB	30	30	0	10
		21 day dB	40	30	0	0
		Threshold shift	10	0	0	-10
	5 MET 2	Baseline dB	30	20	0	0
		21 day dB	30	20	0	0
		Threshold shift	0	0	0	0

SIU 7396
PATENT

5 MET 3	Baseline dB	30	30	10	30
	21 day dB	30	40	10	20
	Threshold shift	0	10	0	0
5 MET 4	Baseline dB	30	30	0	0
	21 day dB	20	40	0	0
	Threshold shift	-10	10	0	0
5 MET 5	Baseline dB	30	30	0	20
	21 day dB	30	40	0	20
	Threshold shift	0	10	0	0

Summary graph of all data:



The summary graph shows means and standard error bars for the ABR threshold shifts for each frequency tested for the control and experimental groups. The stars on the graph indicate that the change was significant at the .05 level or better for that particular measure. The experimental group had significantly smaller ABR threshold shifts than the control group at each frequency tested including 2, 4, 6, and 8 kHz.